

Protocol 7: Miniscreens

1. Material

- 50mM Tris + 10mM EDTA
- RNase
- 0.2M NaOH + 1% SDS
- 3M potassium acetate
- 98% ethanol
- 70% ethanol
- ddH₂O

2. Instruments

- centrifuge
- vortex

3. Experimental procedure

- centrifugation of the overnight culture (1', 13,000rpm)
- discard supernatant
- resuspend pellet in 100µl Tris EDTA + RNase, pH 8.0 and vortex
- add 400µl 0.2 M NaOH + 1% SDS, invert 5-10 times
- add 200µl 0.2 M NaOH, 1% SDS at room temperature, invert carefully
- add 150µl 3 M potassium acetate, pH 4.8, invert
- centrifugation (10', 13,000rpm),
- transfer upper phase into two new 1.5ml tubes
- add 1ml cold 98% ethanol, invert
- centrifugation, (10', 13,000rpm)
- discard supernatant, add 1ml 70% ethanol to the pellet, centrifugation (10', 13,000rpm)
- discard as much supernatant as possible
- incubate at 37°C with open lid for 20'
- resuspend in 40µl ddH₂O, pH 8.0
- incubate at 37°C (10', 550rpm)
- store at -20°C